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Synthesis, Characterization and Fluorescence of Novel Bromazepam Derivatives

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Abstract

New bromazepam derivatives namely; 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine -2-thione and 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one have been prepared and characterized by spectroscopic methods (FT-IR, 1H-NMR, UV-visible and EI-mass). Furthermore, the fluorometric method has been developed to detect bromazepam in biological fluid. The new methods are based on measuring excitation and emission spectra of the reaction of bromazepam with cyanuric chloride in pyridine solution compared with excitation and emission spectra free bromazepam in methanolic solution.

Keywords: Bromazepam • Cyanuric chloride • Characterization • Fluorescence

Introduction

Benzodiazepines are most commonly used to treat anxiety, insomnia, and sleep disorders [1]. They are sedative, hypnotic, muscle relaxant, anticonvulsant, and amnesic [2]. Several analytical techniques for determining benzodiazepines in pharmaceutical and biological fluids have been published [3-6]. There have been reports published on measuring the fluorescence of 1,4-benzodiazepines derivatives and their analytical applications. Thermal heating in acidic solvent, photochemical degradation cyclization to acridines following hydrolysis to benzophenones, or derivatization with phethaldehyde and fluorescamine were all used to create fluorescent species in these studies. Some chemicals native fluorescence in acidic solution has also been discovered. However, the fluorescence of benzodiazepines in neutral and acidic solutions is quite modest. Bromazepam is eliminated from the body by hepatic biotransformation, which results in some active and inactive metabolites. These metabolites are conjugated with glucuronic acid for urine excretion.

3-hydroxy bromazepam and 2-amino-3-hydroxy-5-bromobenzoylpyridine are two recognized metabolites of bromazepam. It has a half-life of 20-30 hours and a suggested dosage of 12 mg per day. Thirty percent of the dosage is eliminated unchanged in the urine [7].

The goal of this work is to create novel bromazepam derivatives, namely7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e][1,4]diazepine-2-thione and 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-[1,4]diazepin-2-one. Furthermore, elemental analysis and traditional spectroscopic techniques were used to characterize them. Furthermore, novel, easy, sensitive, and selective fluorometric techniques for detecting trace levels of bromazepam in biological urine samples are being developed. Fluorometric analysis is a very sensitive and discriminatory method. The developed methods are based on the measurement of excitation and emission spectra in methanolic pyridine solution [8].

Materials and Methods

Instrumentation and materials

All the chemicals and different types of solvents used in the current experiment were extremely pure and came from reputable chemical providers like Merck and Sigma-Aldrich. A Perkin–Elmer 2400 series II analyzer instrument was used to determine the percent of elements such as carbon, hydrogen, and nitrogen. The BRUKER AVANCE 400 MHz spectrometer was used to identify the different types of resonating protons, and ^{1}H NMR spectra were collected. The positions and environment of surroundings protons were identi ied after comparing them to an internal standard, Tetra Methyl Silane (TMS), and then determine coupling constants (J). Electronic spectra were veri ied on a Unicam UV-Vis spectrophotometer. A Mattson 5000 FTIR spectrophotometer was used to record infrared spectra (4000 cm^{-1}-400 cm^{-1}) using KBr

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discs. Mass spectra were performed using the DI-50 (direct inlet) unit of a Shimadzu mass spectrometer type GC/MS-QP5050A [9].

Synthesis

Preparation of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine -2-thione: Bromazepam (3.16 g, 0.01 mol), and P_2S_5 (2.22 g, 0.01 mol) were mixed in xylene (30 ml), in a round-bottomed flask were stirred overnight for 10 hours (Figure 1). TLC was employed to monitor the reaction's progress. The resulting reddish-brown solid was filtered and washed with cold EtOH before being dried in vacuo over anhydrous $CaCl_2$.

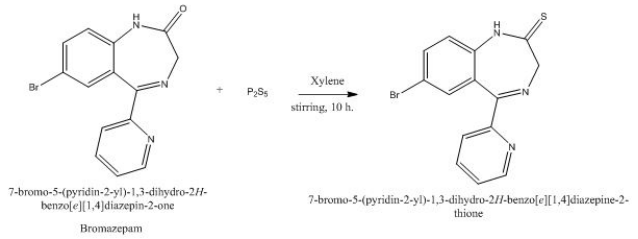

Figure 1. Synthesis of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione.

Preparation of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4] diazepin-2-one: Bromazepam (3.16 g, 0.01 mol), and cyanuric chloride (1.84 g, 0.01 mol) were mixed in pyridine (30 ml), in a round-bottomed flask were stirred overnight for 24 hours. TLC was employed to monitor the reaction's progress [10]. The resulting reddish-brown solid was iltered and washed with cold EtOH before being dried in vacuo over anhydrous $CaCl_2$ (Figure 2).

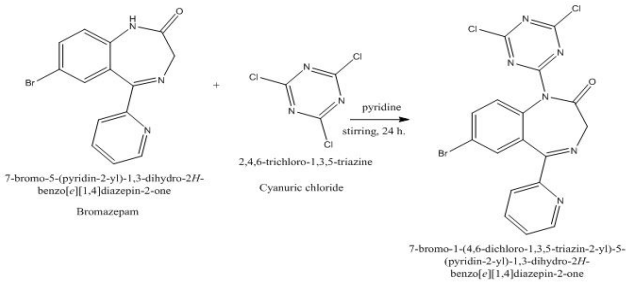

Figure 2. Synthesis of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e][1,4]diazepine-2--thione: Yield 75%; MP: 290ºC, brown solid, FT-IR (KBr cm-1): 1602, 1523, 1309, 1107, 993, 765 and 661; ^{1}H-NMR (DMSO-d6) δ (ppm): 4.62 (s, 2H, CH_2), 7.17-7.50 (m, 3H, Ar-H), 7.6-8.07 (m, 4H, py-H), 9.30 (s, H, NH); Elemental analysis [$C_{14}H_{10}BrN_3S$]: observed (calculated): C 50.45% (50.62%), H 3.14%(3.03%), N 12.33% (12.65%).

7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro -2H-benzo[e] [1,4]diazepin-2-one: Yield 80%; MP: 190ºC, reddish-brown solid, FT-IR (KBr cm-1): 1655sh, 1637, 1594, 1028, 896 and 667; 1H-NMR (DMSO-d6) δ (ppm): 4.42 (s, 2H, CH_2), 6.96-7.30 (m, 3H, Ar-H), 7.42-7.87 (m, 4H, py-H); Elemental analysis [$C_{17}H_9BrCl_2N_6O$]: observed (calculated): C 44.36% (44.00%), H 1.74% (1.95%), N 18.24% (18.11%).

Fluorescence Detection of Bromazepam Using Cyanuric Chloride

Liquid-liquid extraction

Materials: Pure standard of bromazepam were supplied by the National Organization for Drug Control and Research (NODCR). Pharmaceutical preparations, Calmepam tablets containing 1.5 mg of bromazepam were purchased from local market.

Apparatus: pH measurements were carried out using HANNA pH meter HI 8417 with pH sensitivity of ± 0.05 pH units [11].

Reagents: Analytical grades Sodium Carbonate (Na_2CO_3), Potassium Dihydrogen Orthophosphate (KH_2PO_4), Cyanuric chloride and potassium hydroxide were used throughout the whole investigation. Ethyl acetate and absolute ethanol solvents of spectroscopic grades were also used. Double distilled water was used.

Standard solutions: Standard solutions containing 1.581 mg mL^{-1} (5×10^{-3} M) of bromazepam were prepared by dissolving 79.05 mg bromazepam in 50 mL alkaline ethyl acetate. 5×10^{-3} M standard cyanuric chloride solutions in ethyl acetate or in ethanol were freshly prepared each time. 46.1 mg Cyanuric chloride was transferred into 50.0 mL measuring flasks, dissolved in the least amount of solvent and completed to the mark by adding the appropriate solvent.

Extraction: To 5 mL of urine, 2 ml of 0.2 M carbonate buffer (pH 7) were added and mixing followed by 10 ml of ethyl acetate followed by shaking gently for 20 min and then centrifuged at 2500 g for 10 min. Following extraction, the organic layer was transferred to a clean 10 ml glass extraction tube and evaporated to dryness. The residue was dissolved in pyridine and protected from light.

Solid phase extraction

Instrumentation: Centerifuge (Clifton 3200 rpm), vortex (falc), pH-Universal test paper (Macherey-Nagel), Varied micropipette (10-100 µl) and tips (Biohit), vials, Glass tubes (10 ml and 5 ml) and caps, cylinder (10 ml), solid phase extraction system, cartidge C18e Strata, measuring flask (10 ml and 100 ml), analytical balance, and plastic droppers.

Chemicals: Sodium hydroxide, orthophosphoric acid, potassium dihydrogen phosphate, sodium acetate, glacial acetic acid, and ammonium hydroxide.

Solvents: Chloroform, isopropanol, deionized water and methanol.

Reagents

- **Preparation of 0.1 M NaOH reagent:** Add 0.4 g NaOH to measuring flask (100 ml) then add deionized water to make solution up to 100 ml. Store at room temperature.
- **Preparation of 0.1 M KH_2PO_4 reagent:** Add 1.36 g potassium dihydrogen phosphate to measuring flask; add deionized water to make solution up to 100 ml. Store at room temperature.
- **Preparation of phosphate buffer (pH=6):** Add 29 ml of 0.1M NaOH to 50 ml of 0.1 M potassium dihydrogen phosphate.

- **Preparation of acetate buffer (pH=4):** Dissolve 0.41 gm of sodium acetate in 50 ml deionized water then add drops of glacial acetic acid.

Extraction procedure

Urine preparation

- Allow specimens and reagents to equilibrate to room temperature.
- Label analysis vials.
- Add 4 ml of phosphate buffer to 2 ml urine specimen into a glass-tube (10 ml)
- Centrifuge (10 min).
- Vortex (5 min).

Conditioning of SPE Cartridge

- Add 3 ml methanol.
- Add 1 ml phosphate buffer.
- Add 3 ml deionized water.

N.B (We must add step by step and be careful that cartridge must not dried).

Sample load

Add the prepared samples.

Washing

- Add 3 ml deionized water.
- Add 3 ml acetate buffer.
- Add 3 ml methanol.
- Leave cartridge for completely dryness.

Elution

Apply 3 ml from following organic solvent mixture ($CHCl_3$: Isopropanol: Ammonium hydroxide) with ratio (80:19:1)

Stoichiometry procedures: Stoichiometry of Formed 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1, 3-dihydro-2H-benzo[e] [1,4]diazepin-2-one was determined by addition of 1.0 mL of Cyanuric chloride solution (5×10^{-3} M) in the proper extracted urine sample from volunteer containing bromazepam. Fluorescence intensity was measured using Shimadzu RF-540 Spectrofuorometer at the respective emission and excitation wavelengths of bromazepam and extracted urine [12].

Results and Discussion

FT-IR spectra

The FT-IR spectrum of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine -2-thione (Figures 3 and 4) shows band at 1602 cm^{-1} is assignable to υ(C=C). Furthermore, the bands at 1523 cm^{-1} and 661 cm^{-1} attributed to $\upsilon/\delta(C=N)_{py}$, respectively. Also, the bands at 1107 cm^{-1} and 993 cm^{-1} are attributed to (CH_2) wagging and υ(C-C), respectively. Finally, the bands at 1309 cm^{-1} and 765 cm^{-1} were assigned to υ/δ(C=S) [13-17].

The FT-IR spectrum of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one Figure 2 shows bands at 1655_{sh} and 1594 cm^{-1} are assignable to υ(C=O) and υ(C=C), respectively. Moreover, the band at 1637 cm^{-1} and 667 cm^{-1} assigned to $\upsilon/\delta(C=N)_{py}$, respectively. Furthermore, the bands at 1028 cm^{-1} and 896 cm^{-1} are attributed to (CH_2) wagging and υ(C-C), respectively.

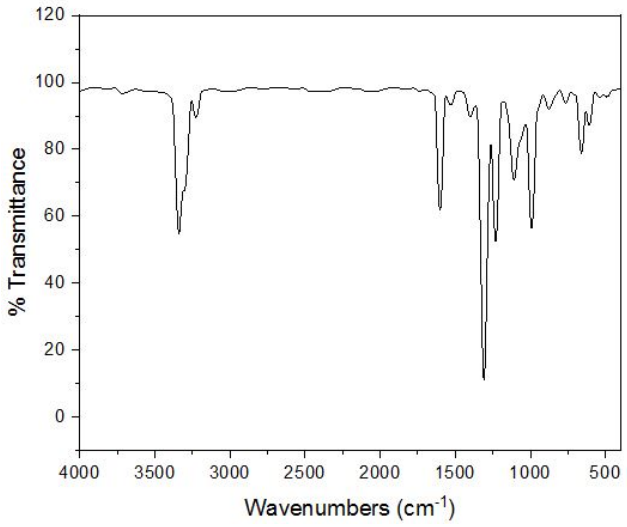

Figure 3. FT-IR spectrum of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine -2-thione.

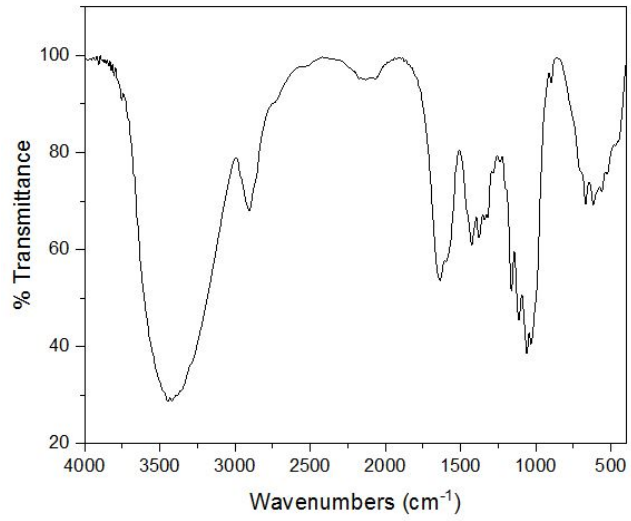

Figure 4. FT-IR spectrum of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl-5-(pyridin-2-yl-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

Electronic spectrum 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl-5-(pyridin-2-yl-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one

The electronic spectrum of 7-bromo-1-(4, 6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo [e] [1,4]diazepin-2-one was recorded in DMSO. The assignments of the significant spectral absorption bands are represented in Figure 5. The 7-bromo-1- (4,6-dichloro-1,3,5-triazin-2-yl -5-(pyridin-2-yl -1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one exhibited bands at 261 and 363 nm, which may

assigned to the n → π* transitions of (C=N) and (C=O) groups, respectively.

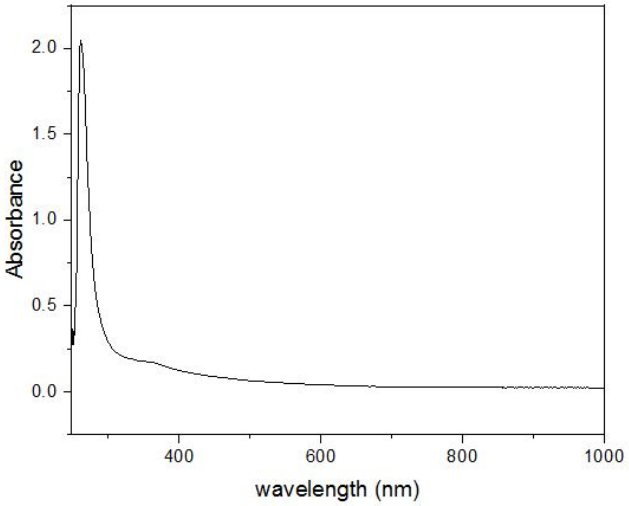

Figure 5. Electronic spectrum of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

^{1}H-NMR spectra

^{1}H-NMR spectrum of the 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione (Figure 4) which shows the signal at 9.30 ppm (singlet, broad) which is assignable to (NH). Moreover, the signal at 4.62 ppm is attributed to the protons of CH_2. The region 7.17-7.50 ppm signals were assigned to aromatic rings protons. Furthermore, the signals at δ=7.60-8.07 ppm were assigned to pyridine ring protons [18].

^{1}H-NMR spectrum of the 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one (Figure 6) which shows the signal at 4.42 ppm is assigned to the protons of CH_2. Furthermore, the region 6.96-7.30 ppm signals were attributed to aromatic rings protons. Finally, the signals at δ=7.42-7.87 ppm were assigned to pyridine ring protons (Figure 7).

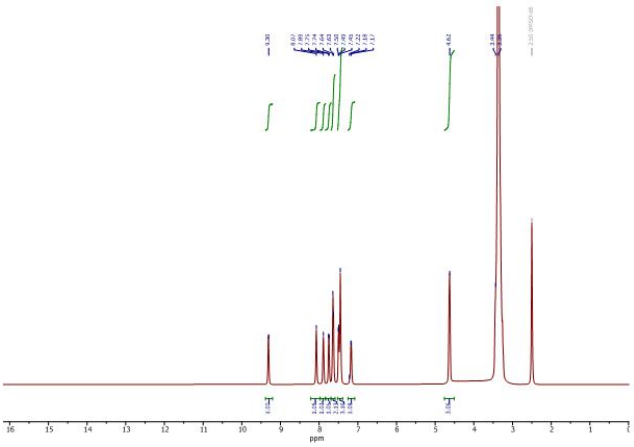

Figure 6. ^{1}H-NMR spectrum of the 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione in d6-DMSO.

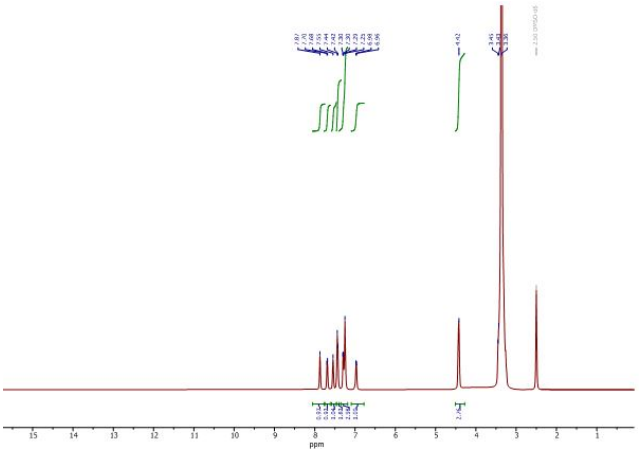

Figure 7. ^{1}H-NMR spectrum of the 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one in d6-DMSO.

Mass spectra

Mass spectra of both prepared compounds (Figures 8-11) shows that their suggested molecular structure as they exhibit molecular ion peaks at m/z: 331.24 and 464.99 which approve their submitted molecular weight. The fragmentation pattern for the 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione and 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5- (pyridin-2-yl)-1,3 dihydro- 2H-benzo [e] [1,4] diazepin-2-one is given. Furthermore, the different fragments of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4] diazepine-2-thione give peaks at different m/z values like: 77.43 (86.12%), 78.63 (15.05%), 227.57 (0.83%), 251.23 (33.89%), and 331.24 (100%), these peaks match (C_5H_3N), (C_5H_4N),($C_{12}H_9N_3S$), ($C_{14}H_9N_3S$) and ($C_{14}H_{10}BrN_3S$) fragments, respectively, while in case of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one, the mass spectrum shows peaks at different m/z values like: 74.02 (100%), 121.30 (79.57%), 163.07 (11.94%), 315.08 (66.17%), 464.99 (21.97%), these peaks match ($CClN_2$), ($C_2Cl_2N_2$), ($C_3Cl_2N_4$),($C1_4H_9BrN_3O$) and ($C_{17}H_9BrCl_2N_6O$) fragments, respectively [19,20].

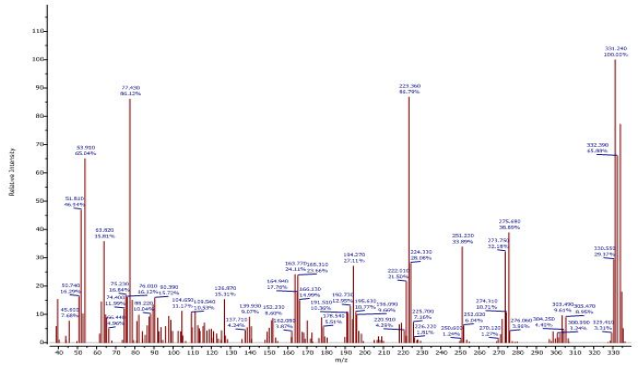

Figure 8. Mass spectrum of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione.

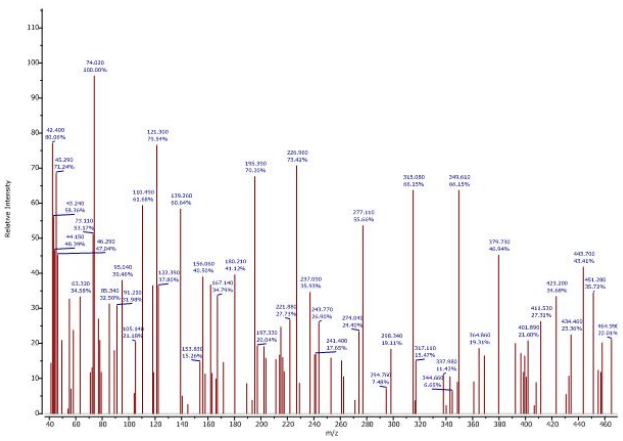

Figure 9. Mass spectrum of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

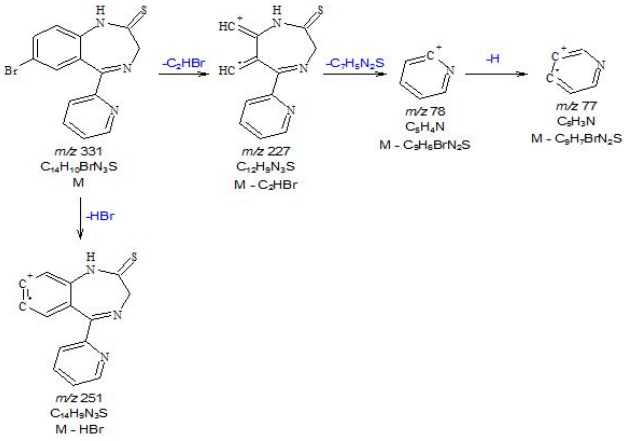

Figure 10. Fragmentation pattern of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepine-2-thione.

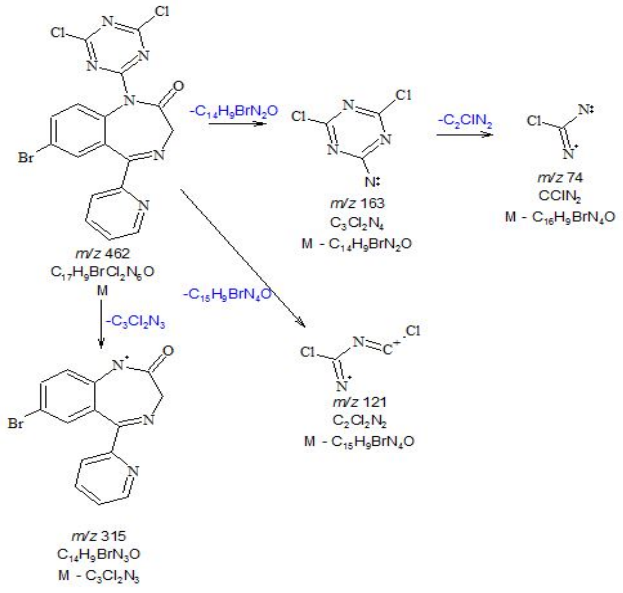

Figure 11. Fragmentation pattern of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

Figure 11. Fragmentation pattern of 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one.

Fluorescence detection of bromazepam using Cyanuric chloride

Firstly, the extraction of bromazepam was con irmed by GC-mass analysis of the residue (Figure 8). Insignificant uorescence emissions were observed for the investigated bromazepam in methanolic/KOH solution compared with the mixture of solution of urine extract and cyanuric chloride. In methanolic KOH, significant uorescence was observed at emission wavelengths of 421 nm and excitation wavelength of 362 nm for bromazepam (Figures 12 and 13). As the reaction of bromazepam with cyanuric chloride, the uorescence spectrum of bromazepam is enhanced with shift of the emission wavelength to 410 nm and shift of excitation wavelength to 384 nm. The reason is attributed to the formation of highly intense 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one as shown.

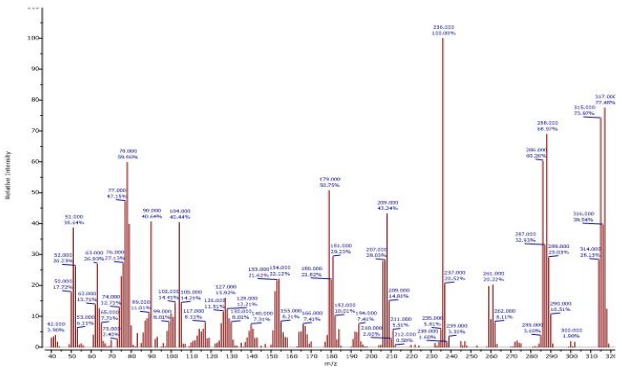

Figure 12. Mass spectrum of bromazepam.

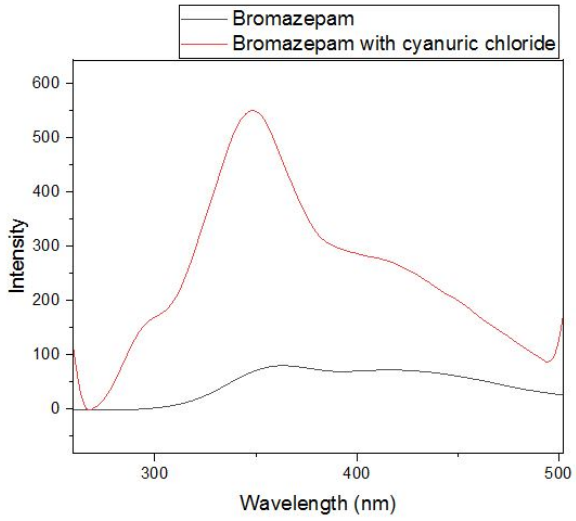

Figure 13. The fluorescence spectra of (a) Bromazepam (b) bromazepam with Cyanuric chloride.

Conclusion

The structure of new bromazepam derivatives namely; 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4] diazepine-2-thione and 7-bromo-1-(4,6-dichloro-1,3,5-triazin-2-yl)-5-(pyridin-2-yl)-1,3-dihydro-2H-benzo[e] [1,4]diazepin-2-one was con irmed by elemental analysis and spectroscopic methods (FT-IR, ^{1}H-NMR, UV-visible and EI-mass). Furthermore, the fluorometric methods have been developed to detect bromazepam in biological luid. The new method shows that the luorescence spectrum of bromazepam is enhanced with shift of the emission wavelength to 410 nm and shift of excitation wavelength to 384 nm by the reaction with cyanuric chloride in pyridine solution.

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